transfer into a 50-milliliter volumetric flask approximately 16 milligrams of clavam-2-carboxylate authentic sample. Dilute to volume and transfer 10 milliliters into a 100-milliliter flask. Dilute to volume with water.

(*b*) *Preparation of sample solution.* Accurately weigh 100 milligrams of the sample into a 10-milliliter flask. Dilute to volume with water.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) for the clavulanate standard peak is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 4,000 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and clavam-2-carboxylic acid peaks is satisfactory if it is greater than 1.0.

(*d*) *Coefficient of variation (Relative standard deviation).* The coefficient of variation (S_R in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.352(b) of this chapter.

(iv) *Calculations.* Calculate the percent of clavam-2-carboxylate content as follows:

$$\text{Percent clavam-2-carboxylate content} = \frac{\text{Mean sample height (or area)} \times \text{weight of standard} \times P}{\text{Mean peak height (or area) of standard} \times \text{weight of sample} \times 50}$$

where:

P=Percent clavam-2-carboxylic acid in the standard.

[49 FR 39674, Oct. 10, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§455.15a Sterile clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1.5 percent.

(v) Its pH of an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(vi) It gives a positive identity test.

(vii) Its [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) content is satisfactory if it is not greater than .01 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content.* Proceed as directed in §455.15(b)(1) of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 10 milligrams per milliliter of clavulanate potassium.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(6) *Identity.* Proceed as directed in §436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(7) *Clavam-2-carboxylate content.* Proceed as directed in §455.15(b)(5) of this chapter.

[50 FR 33519, Aug. 20, 1985, as amended at 54 FR 11584, Mar. 29, 1990]

§455.20 Cycloserine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish compound. It has the

chemical structure D-4-amino-3-isoxazolidone. It is so purified that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in a 10 percent aqueous solution is not less than 5.5 and not more than 6.5.

(v) Its residue on ignition is not more than 0.5 percent.

(vi) It gives a positive identity for cycloserine.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Colorimetric assay*—(*a*) *Stockstandard solution*. Dry approximately 100 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient distilled water to give a solution containing 1,000 micrograms per milliliter. This solution may be used for 1 month if kept under refrigeration.

(*b*) *Standard curve solutions.* Pipette accurately 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 milliliters of the stock standard solution to each of six 100-milliliter volumetric flasks, dilute to 100 milliliters with 0.1*N* sodium hydroxide and mix thoroughly.

(*c*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of 4 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in an amber bottle.

(*d*) *Procedure.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 100 milliliter volumetric flask. Dissolve in sufficient 0.1*N* sodium hydroxide to measure exactly 100 milliliters. Mix thoroughly and transfer 10 milliliters to a second 100-milliliter volumetric flask, and mix thoroughly. Transfer exactly 1.0 milliliter of each of the standard curve solutions and of the sample solution to respective test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid to each of the test tubes. Mix thoroughly. Add exactly 1.0 milliliter of oxidized nitroprusside reagent to each test tube and mix thoroughly. Allow the tubes to stand at room temperature for at least 10 minutes in order that maximum color intensity may develop. Using the solution containing 0.0 milliliter of working standard as a blank, determine the absorbances of the solutions at 625 nanometers in a suitable spectrophotometer. Plot concentration versus absorbance on linear graph paper. The curve may deviate slightly from a straight line. The standard curve solutions equal 0, 10, 50, 100, 150, and 200 micrograms of cycloserine, respectively.

(*e*) Calculations:

Micrograms cycloserine per milligram = (Concentration in micrograms from calibration curve × 1,000)/Weight of original sample in milligrams.

(ii) *Microbiological turbidimetric assay*. Proceed as described in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution with a concentration 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Proceed as directed in paragraph (b)(1)(i) of this section.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.40 Mupirocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mupirocin is nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-l-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]-,[2*S*-[2α(*E*),3*B*,4*B*,5α[2*R**,3*R**(1*R**,2*R**)]]]-. It is a white to off-white crystalline solid. It is so purified and dried that:

(i) Its potency is not less than 920 micrograms per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.0 percent.

(iii) The pH of a saturated aqueous solution of mupirocin is not less than 3.5 and not more than 4.0.

(iv) It is crystalline.

(v) It gives a positive identity test for mupirocin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 229 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as an octadecylsilane, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Use the resolution test solution to determine resolution in lieu of the working standard solution. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Acetonitrile.* Distilled in glass. Ultraviolet grade.

(B) *Phosphate buffer, pH 6.3.* Prepare a 0.05*M* sodium monobasic phosphate solution and adjust to pH 6.3 with 1.0*N* sodium hydroxide.

(C) *Mobile phase.* To 750 milliliters of 0.05*M*, pH 6.3 phosphate buffer, add 250 milliliters of acetonitrile. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution, test solutions*—(A) *Working standard solution.* Accurately weigh approximately 11 milligrams of the mupirocin working standard into a 100-milliliter volumetric flask. Dissolve the standard in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phosphate buffer. Mix well.

(B) *Sample solution.* Transfer approximately 11 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask. Dissolve the sample in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phospate buffer. Mix well.

(C) *Resolution test solution.* Acidify approximately 10 milliliters of the working standard solution with 6*N* hydrochloric acid to pH 2.0. Allow to stand at room temperature for about 2 hours. Neutralize this solution. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows: